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2 Wind-induced collapse of the biopolymeric surface microlayer induces sudden
3 changes in sea surface roughness

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17 **Abstract**

18 All exchange between the ocean and atmosphere has to cross the sea surface microlayer (SML), yet the SML
19 impact on modulating air-sea exchange rates remains poorly understood. Surfactants, including biopolymers, can
20 influence exchange rates by altering the rheological properties of the SML, damping surface turbulence, and
21 capillary wave formation. We investigated the impact of wind speed on SML biopolymer enrichment, surface
22 roughness, and interfacial surfactant coverage at the Heidelberg ‘*Aeolotron*,’ a large annular wind-wave facility
23 filled with 18.000L seawater. Our results show that biopolymer enrichment, specifically the enrichment of
24 polypeptides and polysaccharides, in the SML declined sharply at wind speeds above 6 m/s, coinciding with a
25 sudden increase in the Mean Square Slope (MSS) of waves by 1–2 orders of magnitude. At wind speed $< 6\text{ m s}^{-1}$,
26 biopolymer enrichment in the SML was accompanied by high surfactant surface coverage and strongly reduced
27 MSS values compared to non-enriched or essentially surfactant-free clean freshwater surfaces, indicating a
28 substantial impact of biopolymer enrichment in the SML for air-sea exchange at lower wind speed. Selective SML
29 enrichment was observed, particularly for the amino acids arginine and glutamic acid, and the amino sugar
30 galactosamine. Amino acid and carbohydrate monomers in the SML also exhibited significant and compound-
31 specific wind-induced variability. Our findings suggest that biopolymers, particularly those derived from bacterial
32 production, accumulate in the SML and act as powerful biosurfactants. Unlike artificial surfactant films, natural
33 SML components were more susceptible to wind-induced disruption and to microbial production and
34 decomposition. Our findings reveal that ecological processes actively regulate the chemical and physical properties
35 of the SML, including surfactant surface coverage, and thereby potentially modulate air–sea heat and mass
36 exchange.

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39 **1. Introduction**

40 All exchange between the ocean and atmosphere traverses a thin upper ocean boundary layer known as the sea
41 surface microlayer (SML) (Cunliffe et al., 2013; Engel et al., 2017). Less than 1mm thick, the SML is the
42 chemically and structurally complex organic interface layer right below the air-sea interface with distinct physical,
43 chemical, and biological properties, often enriched in high molecular weight biopolymers and surface-active
44 agents (surfactants). These surfactants are amphiphilic molecules with both hydrophilic (water-attracting) and

45 hydrophobic (water-repelling) groups. Under low-wind conditions, the accumulation of organic material and
46 surfactants in the SML dampens capillary waves and reduces light reflection, making the SML appear smooth, a
47 phenomenon often referred to as a slick. In the ocean, slicks appear shiny, calm, or darker than the surrounding
48 water because they reflect sunlight differently.

49 Various biochemicals, including heteropolymers of lipids, amino acids, and carbohydrates, contribute to the
50 oceanic surfactant pool (Cunliffe et al., 2013, Gašparović and Čosović, 2003). For example, in
51 lipopolysaccharides, the carbohydrate and lipid moieties represent the hydrophilic and the hydrophobic parts of
52 the molecule. Surfactants can impede air-sea gas exchange by modifying the surface rheological properties of the
53 SML. Specifically, surfactants increase the surface elasticity and effective surface viscosity of water. As a result,
54 Marangoni stresses arise from surface-tension gradients. This damps the formation of capillary waves, which
55 reduces small-scale roughness, and leads to a stronger turbulent energy dissipation near the surface (Wei and Wu,
56 1992; Frew et al., 1990; Jenkinson et al., 2018; Laxague et al., 2024). In this context, the overall effect of
57 surfactants arises from complex and dynamic competitive adsorption: an excess of highly surface-active
58 compounds inhibits the adsorption of less active surfactants, while a deficiency promotes the contribution of the
59 latter (Pogorzelski et al., 2006; Frka et al., 2012). In the open ocean, organic matter derived from phytoplankton
60 production contains surfactants (Croot et al., 2007; Frew et al., 1990; Wurl et al., 2011). Regions with elevated
61 primary production are therefore expected to have higher surfactant concentrations (Wurl et al., 2011). However,
62 chlorophyll *a* (Chl *a*), often used as a proxy for primary production, may not accurately predict surfactant
63 occurrence (Laß et al., 2013; Sabbaghzadeh et al. 2017). Instead, a mixture of more recalcitrant dissolved organic
64 matter (DOM) and freshly produced biopolymers seems to control surfactant dynamics in the SML (Barthelmeß
65 and Engel, 2022). Certain strains of heterotrophic bacteria produce surfactants (Satpute et al., 2010) and have also
66 been associated with surfactant-covered ocean surfaces (Kurata et al., 2016). In addition, surfactants present in
67 seawater have been associated with human-related and terrestrial sources, such as riverine runoff (Cuscov and
68 Muller, 2015; Shararom et al., 2018).

69 Variability of surfactants in the SML is likely one of the main reasons why parameterizations based solely on wind
70 speed struggle to accurately predict mass and momentum exchange between the sea and atmosphere, particularly
71 at low wind speeds where the number of observations is small (Wanninkhof et al., 2009; Nagel et al., 2019). This
72 significantly hinders accurate estimates of the ocean's contribution to the cycling of greenhouse gases. For
73 example, a substantial reduction of air-sea fluxes of CO₂ has been documented under high accumulation of natural
74 surfactants using surface seawater of the Atlantic in an on-board air-sea gas exchange tank experiment (Pereira et

75 al., 2018). In association with cyanobacteria blooms (*Trichodesmium* sp.) in the Baltic Sea, a drastic reduction of
76 the gas transfer coefficient (k_w) was associated with bloom-induced biosurfactants, leading to $\pm 20\%$ differences in
77 seasonal CO_2 uptake estimates (Schmidt and Schneider, 2011). Another study in the eastern tropical North Atlantic
78 indicated that surfactants, especially in areas of high biological productivity, may dampen the air-sea exchange of
79 other greenhouse gases like N_2O as well (Kock et al., 2012). Estimates on how surfactants in the SML reduce
80 global net oceanic CO_2 uptake vary between 15% and 60% (Pereira et al., 2018; Asher et al., 1997; Tsai and Liu,
81 2003; Wurl et al., 2016). However, at sea, the variability and complexity of organic matter composition, combined
82 with a dynamic physical environment, including waves, rain, and varying wind speed, make it hard to directly
83 quantify the influence of surfactants on air-sea gas exchange and to examine which biochemical components
84 contribute to the surfactant pool. Repeated conditions of constant wind speeds, especially in the low wind regime,
85 are challenging to meet in the open ocean.

86 To investigate the influence of wind speed and surfactants on air-water mass exchange under more controlled
87 conditions, wind-channel experiments have typically been conducted using freshwater and defined additions of
88 artificial surfactants such as oleyl alcohol, hexadecanol, Triton-X and hexadecylamine (Hühnerfuss et al., 1981;
89 Jähne, 1987, Alpers and Hühnerfuss, 1989; Mesarchaki et al., 2015; Frew et al., 1995; Gade et al., 1998; Krall,
90 2013). These studies demonstrated strong wave damping of surfactants up to a wind speed of 13 m s^{-1} (Broecker
91 et al., 1978; Jähne, 1987). Only a limited number of wind-channel experiments have been conducted using natural
92 surface films and seawater. For example, Tang and Wu (1992) demonstrated the wave-damping capacity of natural
93 films under varying wind speeds, but did not investigate the biochemical composition of the surfactants.
94 Contributing to a joint effort to close this knowledge gap, by conducting an experimental campaign at the
95 Heidelberg *Aeolotron*, a unique large-scale facility capable of generating controlled wind conditions of up to 22
96 m/s, which we filled with 18000L natural seawater. Unlike previous investigations that relied largely on artificial
97 surfactants, freshwater, or simplified laboratory systems, our approach allowed us to directly examine natural
98 marine biochemicals under controlled yet realistic SML conditions. Specifically, we investigated how wind speed
99 influences the enrichment of the two quantitatively most abundant biopolymer classes, total hydrolysable amino
100 acids (THAA) and total combined carbohydrates (TCCHO), in the SML and how these biopolymers contribute to
101 capillary wave damping. To further link SML composition to surface physical properties, we also quantified
102 surface roughness in terms of mean square slope measurements and surfactant surface coverage.

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104 **1. Material and Methods**

105 **2.1 Experimental conditions and treatments**

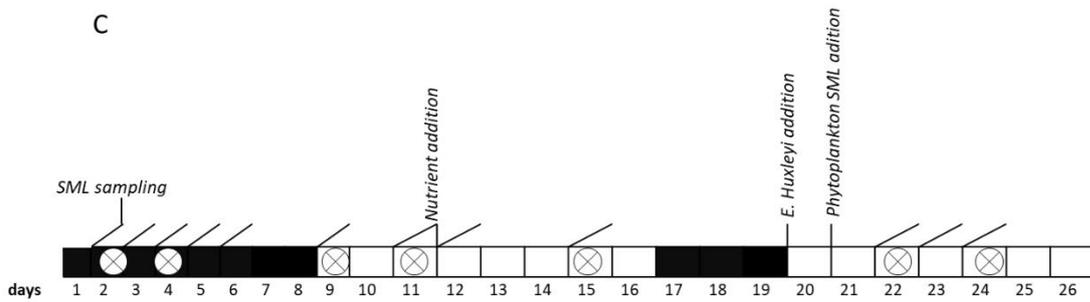
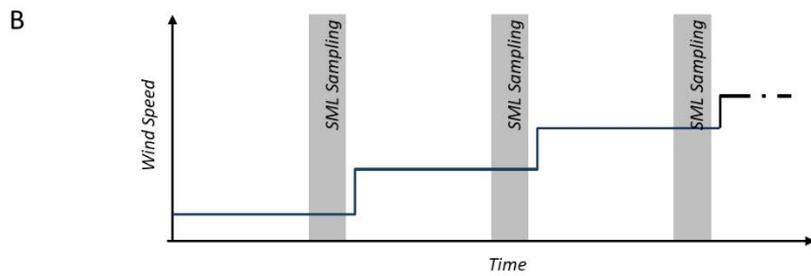
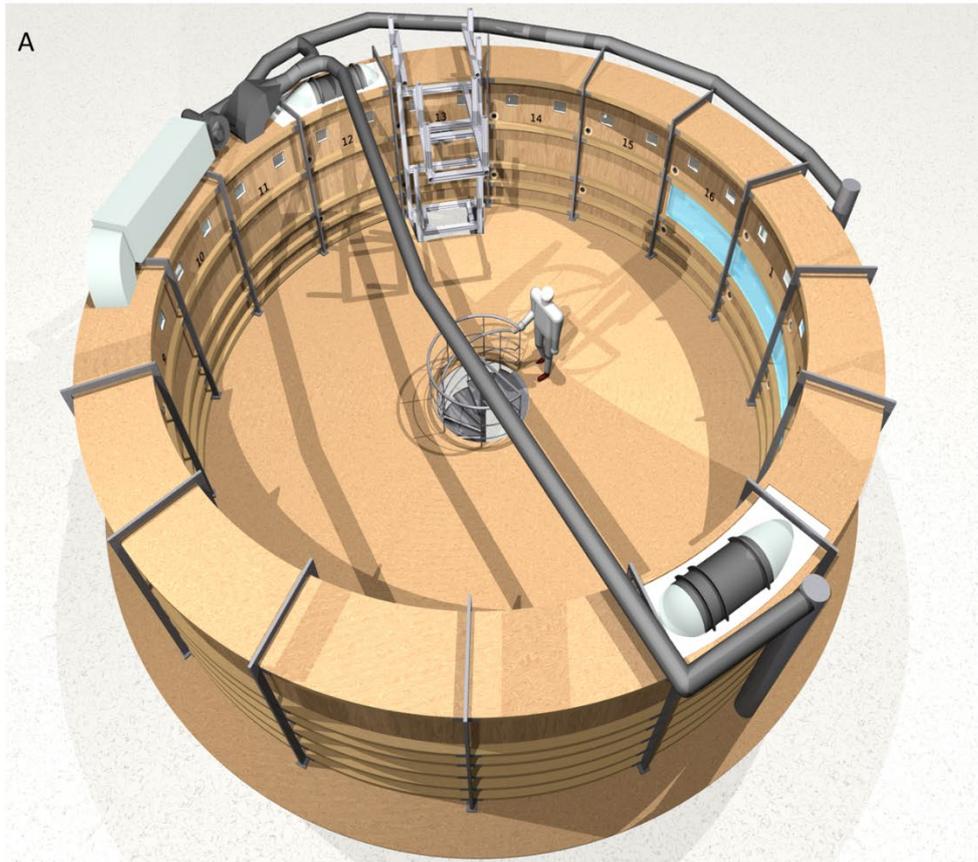
106 This study was part of the larger ‘*Aeolotron*’ experiment, conducted in November 2014 to investigate various air-
107 sea exchange processes under controlled wind conditions. The *Aeolotron* is an annular wind wave tank in
108 Heidelberg, Germany, with a diameter of approximately 10 m, a water depth of 1 m, a 1.4m air space above the
109 water, and a total surface area of 18.4 m² (Figure 1A).

110 Due to its unique annular geometry, the *Aeolotron* wind-wave tank offers distinct advantages over conventional
111 linear wind-wave tanks when aiming to replicate ocean-like conditions (Schmundt et. al, 1995). In linear tanks,
112 surfactants tend to accumulate near the wave absorber and are eventually rendered inactive, as they are transported
113 out of the active measurement region by wind and wave action. In contrast, the annular design of the *Aeolotron*
114 ensures that surface films remain uniformly distributed, allowing for sustained and realistic interactions at the air-
115 water interface. Additionally, while conventional linear tanks are limited by fetch, the *Aeolotron* permits the
116 continuous development of wind waves along an effectively unlimited fetch, allowing for the generation of older,
117 more ocean-like wave fields. This enables the study of processes that are otherwise difficult to capture in shorter
118 linear facilities.

119 Importantly, the limited absolute size of the *Aeolotron* does not compromise its relevance for studying interfacial
120 gas exchange processes. The key mechanisms governing air-sea gas exchange, particularly those involving the sea
121 surface microlayer (SML), operate on length scales of millimetres or less. These include molecular diffusion,
122 micro-scale turbulence, and surfactant-mediated suppression of short capillary waves, all of which are fully
123 resolved within the *Aeolotron*'s experimental framework (Schmundt et al., 1995; Mesarchaki et al., 2015). As such,
124 the *Aeolotron* provides an excellent platform for investigating the fundamental physics of gas transfer and
125 interfacial dynamics under highly controlled yet ocean-relevant conditions.

126 The setup of the *Aeolotron* experiment and the physical, chemical and biological treatments in the course of the
127 experiment are described in more detail elsewhere (Engel et al., 2018). Briefly, the wind wave channel was filled
128 with approximately 18000 L of seawater, which had been collected in September 2014 in the North Atlantic and
129 German Bight, North Sea. The seawater had been stored in the dark at 10°C for about a month until it was used to
130 fill the *Aeolotron*.

131



132

133 **Figure 1, A-C: Top-view on the Heidelberg annular wind-wave channel *Aeolotron* (A), where experiments**
134 **with increasing wind speed were conducted on seven days. Step-wise increase in wind speed applied**
135 **during each wind experiment (B) and timeline of the *Aeolotron* study with different seawater**
136 **modifications and the seven wind experiments (crossed circles) (C).**

137 Seawater temperature within the *Aeolotron* ranged from 20.13 to 22.21°C. Light sources were operated over the
138 tank for two periods of eight (days 7-16) and six days (days 20-26), providing a photon flux of 115-120 $\mu\text{mol m}^{-2}$
139 s^{-1} . Inorganic nutrients were added on day 12. About 800 ml of a culture of *Emiliana huxleyi* (cell density: $4.6 \times$
140 $10^5 \text{ cell ml}^{-1}$) was added on day 20. In addition, 6L of biogenic microlayer sampled with the glass plate during a
141 previous phytoplankton mesocosm experiment, stored frozen at -20°C for about 6 months, was thawed and added
142 on day 21. The total duration of the *Aeolotron* experiment was 26 days.

143

144 During the *Aeolotron* experiment, a total of 7 wind experiments were conducted on days 2, 4, 9, 11, 15, 22, and
145 24 (Figure 1B, C). During each experiment, the wind speed was increased stepwise yielding a range of wind speeds
146 (U_{10}) from 1.3 m/s to 21.9 m/s. The duration of each wind speed setting varied from 30 min to 2 hrs, with longer
147 durations for the lower wind speeds. This scheme was chosen to facilitate robust concurrent measurements of air-
148 sea gas exchange for each wind speed condition in a parallel project (Mesarchaki et al., 2015).

149 Wind speeds during the experiments were measured using a Pitot tube, and water velocities were measured using
150 an acoustic Doppler velocimeter mounted equidistant from both the outer and inner wall at a water depth of
151 approximately 50 cm. The friction velocity U_* , a measure for the wind's momentum input into the water, was
152 calculated from the water velocity using a momentum balance method (Bopp, 2014). The friction velocity U_*
153 measured in the *Aeolotron* was subsequently converted to U_{10} using a parametrization of the drag coefficient
154 derived from the open ocean (Edson et al., 2013).

155

156 **2.2 Wave Slope Measurement**

157 The Mean Square Slope (MSS, a statistical dimensionless parameter for surface roughness) of the water surface is
158 strongly correlated with the air-sea gas transfer velocity (Jähne et al., 1987; Frew et al., 2004). The MSS is,
159 therefore, an important parameter linking sea surface properties to air-sea exchange processes. During this study,
160 the MSS of wind-induced waves was computed from wave slope images (Kiefhaber et al., 2014). These images
161 were taken by a high-speed camera, positioned in a telecentric setup above the water surface, capturing images of
162 a wave-height independent area at the water surface measuring 16 cm x 20 cm, achieving a resolution of 0.22
163 mm^2/pixel at a rate of 1500 frame per second. Illumination of the water body was achieved from below, utilizing
164 a programmable high-power LED light source in such a way that both the along-wind and cross-wind slopes, s_x
165 and s_y of the waves, could be computed. From the slope images, the MSS is simply computed as an average over
166 space and time, $\text{MSS}=(s_x^2 + s_y^2)$. As a reference, the variation of MSS with wind speed was determined for clean

167 freshwater in a separate *Aeolotron* study beforehand (Kunz, 2017). Uncertainties for MSS values are <10% for
168 values >0.002. Close to the detection limit of 0.0003, uncertainties are in the order of the measured value.

169

170 **2.3 Sampling**

171 The SML was sampled on 12 days in the morning at low wind speed (U_{10} : 1.3-1.5 m s⁻¹) and towards the end of
172 each wind speed step during each of the seven wind experiments (Figure 1B). Sampling was carried out using the
173 glass plate technique in accordance with established protocols (Cunliffe and Wurl, 2014), employing a borosilicate
174 glass plate (500 × 250 × 5 mm) and a Teflon wiper. For sampling, the glass plate was inserted perpendicular to the
175 surface and withdrawn at a rate of ~20 cm/sec. Subsequently, the sample, retained by surface tension, was removed
176 utilizing a Teflon wiper. Each sampling involved between 23 and 48 dips and precise documentation of the number
177 of dips and total volume collected. All samples were collected in acid-cleaned (10% HCl) glass bottles, washed
178 with ultrapure water from a Milli-Q system, and rinsed with 20 mL of sample initially. Before each sampling
179 event, both the glass plate and wiper were cleaned with 10% HCl and extensively rinsed with Milli-Q water.

180 The thickness (d , μm) of the SML sampled with the glass plate was approximated:

$$181 \quad (1) \quad d = V / (A \times n)$$

182 where V represents the collected SML volume (ranging from 200-420 mL), A denotes the sampling area of the
183 glass plate ($A = 2000 \text{ cm}^2$), and n is the number of dips (Cunliffe and Wurl, 2014). In this study, d serves as an
184 operational estimate for the thickness of the SML and is referred to as apparent SML thickness.

185 Underlying water (ULW) samples were taken in the morning at low wind speed from a tap ~50 cm below the water
186 surface, representing half the water column's height. These samples, ~500 ml each, were filled into 10% HCl-
187 cleaned borosilicate glass bottles, rinsed with Milli-Q water, and pre-rinsed with ~20 mL of the sample directly
188 before filling. ULW samples were collected daily between day 1 and day 26 of the experiment, except for day 6
189 (Figure 1C).

190 **2.4 Analysis of organic compounds**

191 **2.4.1 Dissolved organic carbon (DOC)**

192 Samples for DOC (20 ml) were collected in duplicate from the SML and ULW and filled into combusted glass
193 ampoules after filtration through combusted glass-fibre filters (GF/F) filters (8 hours, 500° C). Samples were
8

194 acidified with 80 μL of 85% phosphoric acid, heat sealed immediately, and stored at 4°C in the dark until analysis.
195 DOC samples were analyzed by applying the high-temperature catalytic oxidation method (TOC -VCSH,
196 Shimadzu) (Engel and Galgani, 2016). The instrument was calibrated every 8-10 days by measuring standard
197 solutions of 0, 500, 1000, 1500, 2500 and 5000 $\mu\text{g C L}^{-1}$, prepared from a potassium hydrogen phthalate standard
198 (Merck 109017). Every measurement day, Milli-Q water was used to determine the instrument blank, which was
199 accepted for values $<12 \mu\text{g C L}^{-1}$. DOC analysis was validated on every measurement day with deep seawater
200 reference (DSR) material provided by the Consensus Reference Materials Project of RSMAS (University of
201 Miami) yielding values within the certified range of 42-45 $\mu\text{mol C L}^{-1}$. Additionally, two internal standards with
202 DOC within the range of those in samples were prepared each measurement day using a potassium hydrogen
203 phthalate (Merck 109017). DOC concentration was determined in each sample from 5 to 8 injections. The precision
204 was $<4\%$, estimated as the relative standard deviation of replicate measurements.

205 **2.4.2 Biopolymers**

206 Total hydrolysable amino acids (THAA), i.e., amino acids with a peptide bond, including amino acids contained
207 in polypeptides or heteropolymers, like lipopeptides and glycopeptides, were determined in ULW and SML
208 (Lindroth and Mopper, 1979; Dittmar et al, 2009). 5 mL of sample were filled into pre-combusted glass vials (8
209 hours, 500°C) and stored at -20°C until analysis. Duplicate samples were hydrolyzed for 20h at 100°C with HCl
210 (30% suprapur, Merck) and neutralized by acid evaporation under vacuum in a microwave at 60°C. Samples were
211 washed with Milli-Q water to remove the remaining acid. Analysis was performed on a 1260 HPLC system
212 (Agilent). Thirteen different amino acids were separated with a C18 column (Phenomenex Kinetex, 2.6 μm , 150
213 x 4.6 mm) after in-line derivatization with o-phthalaldehyde and mercaptoethanol. The following standard amino
214 acids were used: aspartic acid (ASX), glutamic acid (GIX), serine (SER), arginine (ARG), glycine (GLY),
215 threonine (THR), alanine (ALA), tyrosine (TYR), valine (VAL), phenylalanine (PHE), isoleucine (ILEU), leucine
216 (LEU), γ -aminobutyric acid (GABA). α -aminobutyric acid was used as an internal standard to account for losses
217 during handling. Solvent A was 5% Acetonitrile (LiChrosolv, Merck, HPLC gradient grade) in
218 Sodiumdihydrogenphosphate (Merck, suprapur) Buffer (PH 7.0), Solvent B was Acetonitrile. A gradient was run
219 from 100% solvent A to 78% solvent A in 50 minutes. The detection limit for individual amino acids was 2 nmol
220 monomer L^{-1} . The precision was $<5\%$, estimated as the relative standard deviation of replicate measurements.
221 Based on THAA measurement, the Degradation Index (DI) was calculated as an indicator of the diagenetic status
222 of organic matter (Dauwe and Middelburg, 1998). For instance, leucine typically exhibits preferential
223 degradation compared to glycine. Mole percentages of amino acid were standardized using averages, and

224 standard deviations and multiplied with factor coefficients based on Principal Component Analysis (PCA) as
225 given in Dauwe et al. (1999). Lower DI values indicate more degraded organic matter, whereas higher DI values
226 indicate more fresh organic matter.

227

228 Total hydrolysable carbohydrates > 1 kDa (TCHO), i.e., carbohydrates with a glycosidic bond, including
229 carbohydrates contained in polysaccharides and heteropolymers like glycolipid and glycopeptides, were
230 determined in bulk seawater and in the SML. 20 mL were filled into pre-combusted glass vials (8 hours, 500 °C)
231 and kept frozen at -20 °C until analysis. The analysis was conducted by applying high-performance anion exchange
232 chromatography coupled with pulsed amperometric detection (HPAEC-PAD) on a Dionex ICS 3000 (Engel and
233 Händel, 2011). Samples were desalinated by membrane dialysis (1 kDa MWCO, Spectra Por) for 5 h at 1 °C,
234 hydrolyzed for 20 h at 100°C with 0.4 M HCl final concentration, and neutralized through acid evaporation under
235 vacuum and nitrogen atmosphere (1h, 60 °C). Two replicate samples were analyzed. For our system, the best
236 resolution of sugars was obtained at 25 °C and, therefore, applied constantly during all analyses. In order to
237 minimize degradation of samples before analysis, the temperature in the autosampler was kept at 4 °C. The system
238 was calibrated with a mixed sugar standard solution including the neutral sugars: fucose (4.6 µM, FUC), rhamnose
239 (3.1 µM, RHA), arabinose (2.0 µM, ARA), galactose (2.4 µM, GAL), xylose/ mannose (3.1 µM, XYL/ MAN),
240 glucose (2.4 µM, GLC), amino sugars: galactosamine (2.0 µM, GAL-N), glucosamine (2.8 µM, GLC-N), and
241 acidic sugars: galacturonic acid (2.8 µM, GAL-URA), gluconic acid (5.1 µM, GLC-AC), glucuronic acid (3.0 µM,
242 GLC-URA) and muramic acid (1.9 µM, MUR-AC). Regular calibration was performed by injecting 12.5 µl, 15.0
243 µl, 17.5 µl and 20 µl of mixed standard solution. The linearity of the calibration curves of individual sugar
244 standards was verified in the concentration range 10 nM-10 µM. Therefore, the standard mixture was diluted 10,
245 20, and 50-fold with Milli-Q water. The injection volume for samples and for the blank was 17.5 µl. To check the
246 performance of carbohydrate analysis and stability of the HPLC-PAD system, a 17.5 µl standard solution was
247 analyzed after every second sample. The detection limit was 10 nmol L⁻¹ for each sugar, with a standard deviation
248 between replicate runs of <2%. Milli-Q water was used as a blank to account for potential contamination during
249 sample handling. Blanks were treated and analyzed in the same way as the samples. Blank concentration was
250 subtracted from the sample concentration if above the detection limit.

251 The relative concentration of a substance (A) in the SML was compared to its concentration in ULW by the
252 enrichment factor (EF):

253

$$(4) \quad EF = (A)_{SML} / (A)_{ULW}$$

254 Because of normalization, EFs for different components can be readily compared. Enrichment of a component is
 255 indicated by $EF > 1$, depletion by $EF < 1$. Statistical analyses were conducted using SigmaStat 4.0.

256

257 2.4.3 Surfactant Coverage and Enrichment

258 Samples for surfactant coverage (sc) were taken only for the initially low and at the highest wind speed. Duplicate
 259 50 mL SML samples were collected on 7 experimental days (days 2, 4, 9, 11, 15, 22, and 24) for initially low and
 260 at the highest wind speed; on day 2, only a low wind sample was available. The SML samples were transferred
 261 into polypropylene bottles, immediately frozen at -40°C for transport, and stored at -80°C before analysis using
 262 surface-sensitive non-linear vibrational sum-frequency generation (VSFG) spectroscopy with a commercial
 263 picosecond VSFG spectrometer (EKSPLA, 532 nm up-conversion wavelength). The use of VSFG spectroscopy
 264 for SML surfactant analysis and its interpretation has been shown previously (Engel et al., 2018; Laß and
 265 Friedrichs, 2011). The VSFG signal intensity $I_{\text{VSFG, SML}}$ (integrated over the spectral wavenumber range of C-H
 266 bond signatures, $2750\text{ cm}^{-1} - 3000\text{ cm}^{-1}$) can be related to the surfactant surface coverage via a square root
 267 relationship ($\sqrt{I_{\text{VSFG, SML}}}/\sqrt{I_{\text{VSFG, DPPC}}} \propto sc$), where $I_{\text{VSFG, DPPC}}$ refers to the intensity of a well-defined reference
 268 surfactant monolayer, here an artificial monolayer of the phospholipid dipalmitoylphosphatidylcholine (DPPC), a
 269 well-characterized and chemically stable model surfactant. In our previous work, which focused on the correlation
 270 of low wind speed data with the concentration of γ -aminobutyric acid (GABA) as an indicator for microbial
 271 decomposition (Engel et al., 2018), we have used a highly compressed monolayer of DPPC in its solid 2D phase
 272 as the $\sqrt{I_{\text{VSFG, DPPC}}}$ reference signal for a completely surfactant-covered surface. However, as the complex mixture
 273 of biosurfactants will prevent the formation of such a highly ordered monolayer, we now have adopted the onset
 274 of the DPPC 2D phase transition

275 In order to convert sc into an effective concentration measure for surfactants in the SML and thus enable a direct
 276 correlation with the measured concentration trends of the DOM fractions THAA and TCHO, the exact composition
 277 and surfactant properties of the substances present in the SML would have to be known. However, for a surfactant
 278 pool typically dominated by wet (i.e., “soluble” in contrast to “unsoluble” dry) surfactants (Laß and Friedrichs,
 279 2011), it is reasonable to assume an adsorption equilibrium of bulk SML surfactants with the air-water interface
 280 such that sc can be described by a reduced Langmuir isotherm (Burrows et al., 2014) according to:

281
$$(2) \quad sc = \frac{c^*}{1-c^*} \quad \text{or} \quad (3) \quad c^* = \frac{sc}{1-sc}$$

282 Here, c^* is the reduced concentration $c^* = c/c_{1/2}$ with $c_{1/2}$ corresponding to the effective bulk SML surfactant
283 concentration yielding a half-covered surfactant monolayer. Accordingly, sc increases linearly with c^* at low
284 surfactant concentrations but levels out towards the limiting value of a completely covered surface at high
285 surfactant concentrations. While the surfactant indices c^* and sc derived from the VSFG measurements provide
286 semi-quantitative insights into surfactant abundance and surface coverage, it is important to note that they are
287 based on assumptions and approximations and should be interpreted accordingly. For example, the analysis may
288 be biased by the variable composition of the surfactant pool during the *Aeolotron* study. This may have induced
289 more or less pronounced variations in the effective $c_{1/2}$ value, which, however, was assumed to be constant.

290

291

292 1. Results

293 3.1 Organic matter variations in the SML in the course of the *Aeolotron* experiment

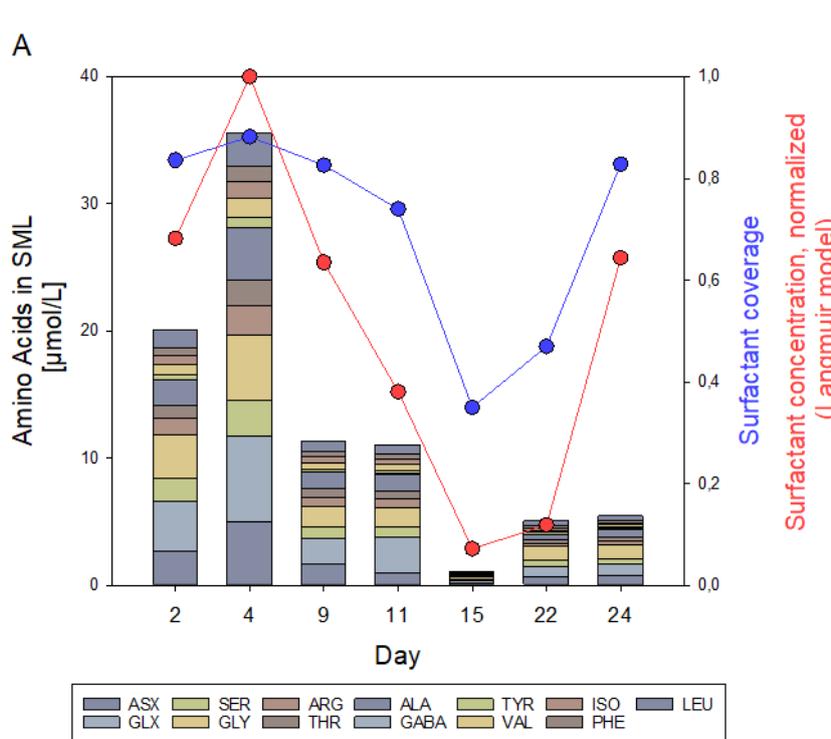
294 Biomass in the water column and variations in microbial abundance and organic matter composition in the course
295 of the *Aeolotron* experiment have been reported previously. To illustrate the conditions in which the wind
296 experiments were conducted, we briefly describe the relevant findings here. Particulate organic matter remained
297 low throughout the experiment, with particulate organic carbon (POC) concentrations ranging between 4 and 29
298 $\mu\text{mol L}^{-1}$. Chlorophyll *a* (Chl *a*) concentration increased after introducing an *Emiliana huxleyi* culture on day 19,
299 reaching peak values of $0.042 \mu\text{g L}^{-1}$ on day 25. DOC concentration in the bulk seawater increased during the
300 course of the experiment (days 2-24) from $85 \mu\text{mol L}^{-1}$ to $120 \mu\text{mol L}^{-1}$. Biopolymers accumulating in the SML
301 can be dissolved, colloidal and particulate. In particular, gel-like particles containing amino acids -Coomassie
302 stainable particles (CSP) and carbohydrate containing transparent exopolymer particles (TEP)- have been shown
303 to accumulate in the SML (Sun et al., 2018). To account for these components in our analysis, and given that the
304 cellular biomass was generally low, we here report total concentrations of the biochemicals, where THAA ranged
305 from 0.83 to $1.67 \mu\text{mol L}^{-1}$ and TCHO from 0.66 to $1.28 \mu\text{mol L}^{-1}$.

306 Throughout the *Aeolotron* experiment, the SML consistently showed enrichment in DOC, THAA, and TCHO,
307 except on day 15, where the difference between SML and ULW fell within analytical error limits. DOC enrichment
308 factors (EF_{DOC}) ranged from 1.0 to 1.6. THAA concentration in the SML was highest on day 4 with $35.5 \mu\text{mol L}^{-1}$,
309 declined to the lowest concentration on day 15 ($1.05 \mu\text{mol L}^{-1}$), and increased again after the addition of natural

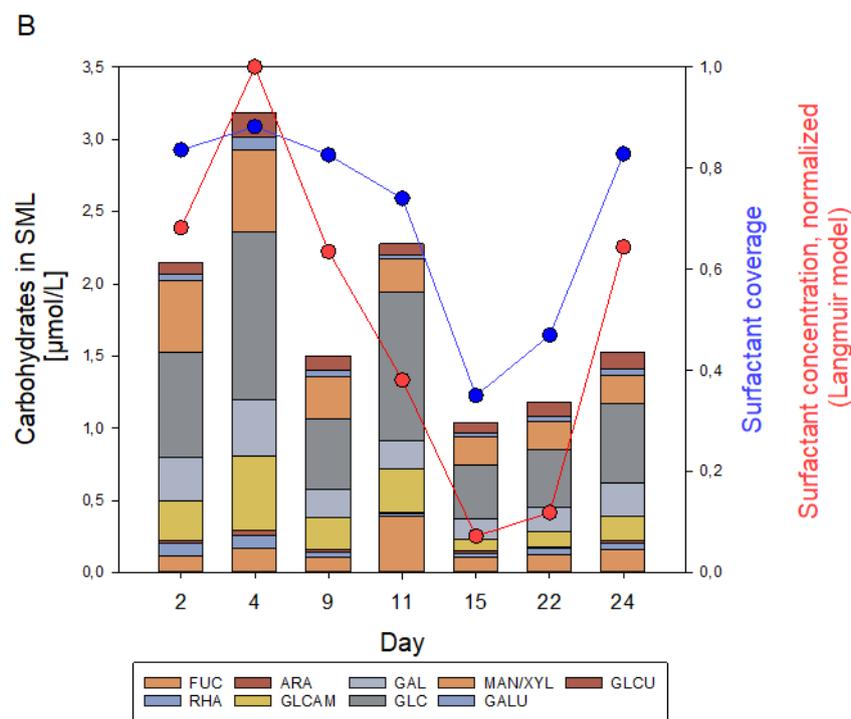
310 phytoplankton-derived organic matter on days 20 and 21 (Figure 2A). In general, the monomeric composition of
 311 THAA in the SML was dominated by GLX (15.8- 25.3 Mol%), GLY (14.2- 21.5 Mol%), and ASX (8.84 – 16.0
 312 Mol%). GABA, an indicator for bacterial degradation, was highest on day 15 with 0.41 Mol%. High enrichment
 313 of THAA in the SML was observed during the first 11 days of the *Aeolotron* experiment ($EF_{THAA}=13-38$). THAA
 314 were slightly depleted in the SML on day 15 ($EF_{THAA}=0.91$) and became enriched again thereafter ($EF_{THAA}=2.89-$
 315 3.24). A selective enrichment of individual amino acids in the THAA pool of the SML was observed, with the
 316 highest enrichment observed on day 4 for the basic amino acid ARG ($EF_{ARG}=74.4$), which contributed only 2.8-
 317 6.2 % Mol to the THAA pool, and for the acidic amino acid GLX ($EF_{GLX}=53.7$).

318 Organic matter accumulating in the SML was generally less degraded than in the ULW, as indicated by the
 319 degradation index (DI) based on THAA composition (Figure S1). This, in turn, suggested that it was the ‘fresher’
 320 fraction of biopolymers that became selectively enriched in the SML. In particular, DI values for organic matter
 321 in the SML were lowest on day 15, when biopolymer concentration was also lowest, indicating preferential
 322 decomposition of the more labile organic matter.

323



324



325

326 **Figure 2A, B: Concentration/composition of (a) THAA and (b) THCO in the SML at low initial wind speed**
 327 **(1.3-2.0 ms⁻¹) and variation of surfactant surface coverage (*sc*, blue circles), as well as normalized reduced**
 328 **bulk SML surfactant concentration (c^*/c_{max} , red circles). Based on a dataset first published in Engel et al.**
 329 **(2018)**

330 TCHO in the SML varied between 2.14 and 1.03 μmol L⁻¹, and -similar to THAA- were higher during the first 11
 331 days of the experiment, lowest on day 15, and increased again until day 24, but without reaching the high values
 332 from the first days of the experiment (Figure 2B). TCHO were enriched in the SML with EF_{TCHO} of 1.5–5.6, with
 333 higher values observed during the first four days of the experiment. TCHO composition in the SML was dominated
 334 by GLC (33-45 Mol%), XYL/MAN (10-23%), and GAL (8.8-15 Mol%). FUC has been considered an indicator
 335 of labile, phytoplankton-derived TCHO (Engel et al., 2012). FUC was 5 Mol% at the beginning of the experiment
 336 and increased after the addition of the phytoplankton-derived material to 17 Mol%. Likewise, GLC-N, as an
 337 indicator of more degraded TCHO, decreased from 12.6 Mol% initially to 8 Mol%. Within the pool of TCHO in
 338 the SML, the highest enrichment was observed on day 4 for the amino-sugar GLC-N ($EF_{GLC-N}=12.89$) and the
 339 acidic sugars GAL-URA and GLC-URA ($EF_{GAL-URA}= 6.70$, $EF_{GLC-URA}= 6.57$). On day 22, biopolymer
 340 concentration in the SML had increased again as natural slick material was added on day 21, yielding EF_{TCHO}
 341 values around 3 on days 22 and 24.

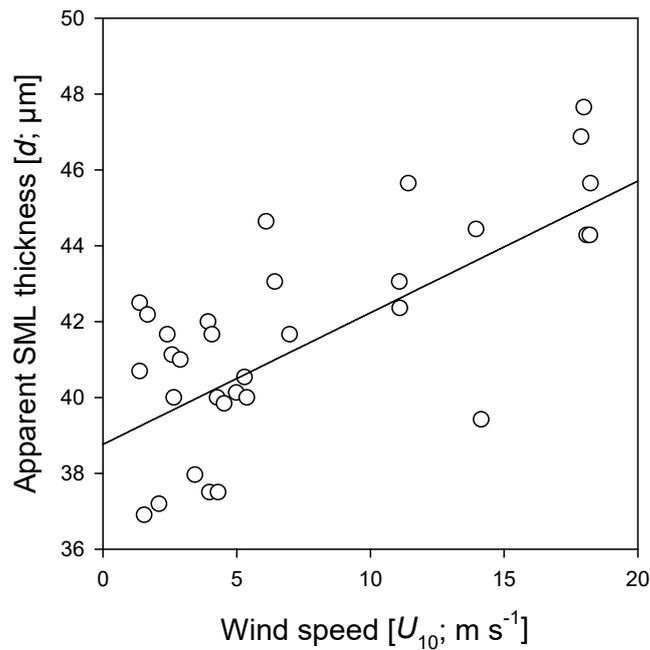
342 The biopolymer ratio [THAA]:[TCHO] was highest on days 2 and 4 with values of 9.4 and 11, respectively, and
343 decreased thereafter. [THAA]:[TCHO] was lowest on day 15 with equal concentrations and yielded 4.3 and 3.6
344 on days 22 and 24.

345 Variations in biopolymers in the SML aligned well with the surfactant surface coverage index sc . Surface coverage
346 was generally high, with $sc > 0.74$ for 5 out of 7 days. The overall similar trend in biopolymer and surfactant
347 abundance was even more evident in the reduced surfactant-concentration data, which have been normalized to
348 the maximum value of $c^* = c_{\max}$ measured on day 2 for clarity. The correlation between the normalized reduced
349 surfactant concentration c^*/c_{\max} and THAA was slightly higher ($r = 0.84; n = 7; p = 0.019$) than for TCHO ($r =$
350 $0.79; n = 7, p = 0.034$). Together with the higher abundance of THAA and presumably higher surface activity of
351 polypeptides compared to polysaccharides (Burrows et al., 2014), this is another indication that, in particular,
352 protein-rich material was important for the formation of the highly surfactant-covered air-water interface.

353

354 3.2 Sea surface properties and biochemical SML composition at increasing wind speed

355 During the *Aeolotron* study and all seven wind experiments, the water column was covered by an SML, with an
356 apparent thickness (d) of 31 - 50 μm . In the course of the *Aeolotron* experiment, SML thickness increased from
357 $d=36\mu\text{m}$ on day 2 to $d=45\mu\text{m}$ on day 24, determined at low wind speed for each measurement day. Combining all
358 data from wind speed experiments days 4 to 24 showed clear patterns regarding the relationship between SML
359 thickness and wind speed (Figure 3). Overall, d increased gradually and significantly with wind speeds ($r=0.63,$
360 $n=34, p < 0.001$). The value of d varied between the same wind speed of different experiments, but was always
361 lowest at low wind speeds, suggesting that SML disruption and mixing between the SML and ULW and surface
362 accumulation of organic components during high wind speeds had no long-lasting (>24hrs) memory effects on
363 SML thickness.



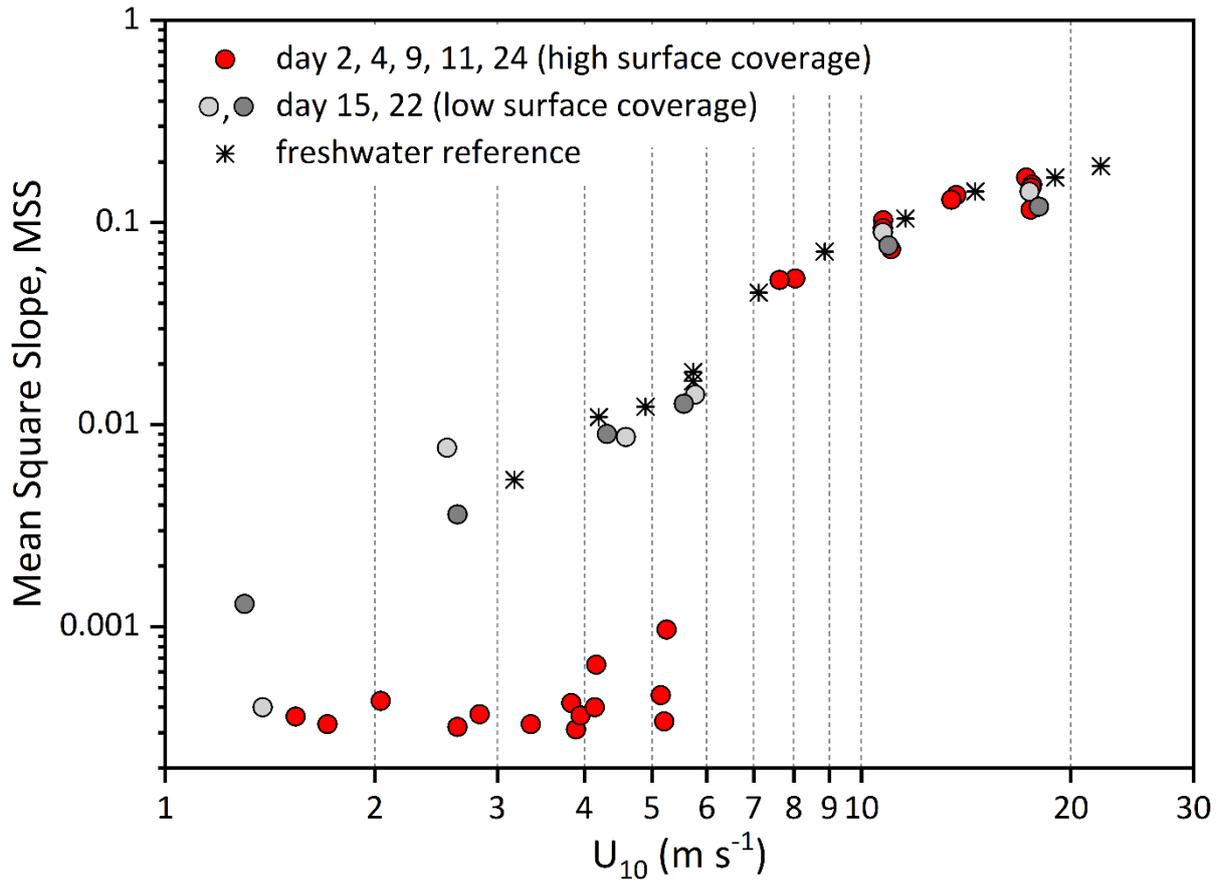
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365 **Figure 3: Relationship between wind speed (U_{10}) and the apparent thickness of the SML (d) as assessed by**
 366 **glass-plate sampling during the *Aeolotron* experiment.**

367

368 The surface roughness, a measure of the small- to medium-scale structure of the wave field that controls how the
 369 sea interacts with wind and light, has been determined by means of the MSS value, a parameter directly correlated
 370 with air-sea exchange of gases and heat. Reference freshwater MSS values showed a gradual increase with wind
 371 speed (U_{10}) from 5.35×10^{-3} at 3.2 m s^{-1} to 1.90×10^{-1} at 22.1 m s^{-1} (Figure 4). The total range of MSS values for
 372 natural seawater was $3.0 \times 10^{-4} - 1.67 \times 10^{-1}$. Compared to freshwater, MSS values abruptly changed around 6 m
 373 s^{-1} and were about 1-2 orders of magnitude lower at wind speeds of $U_{10} < 6 \text{ m s}^{-1}$ during experiments conducted on
 374 days 2, 4, 9, 11 and 24. This strong wave-damping effect at $U_{10} < 6 \text{ m s}^{-1}$ was accompanied by high surfactant
 375 surface coverage values of $sc > 0.74$. On days 15 and 22, with $sc < 0.47$, surface coverage was significantly lower,
 376 and the corresponding MSS values at low windspeed were clearly higher and close to those observed for
 377 freshwater, yielding values of $1.32 \times 10^{-3} - 3.52 \times 10^{-3}$. At wind speeds $> 6 \text{ m s}^{-1}$, MSS generally continued to increase
 378 with wind speed for all available natural seawater samples and, despite some variability between the experimental
 379 days, closely followed the freshwater trend.

380



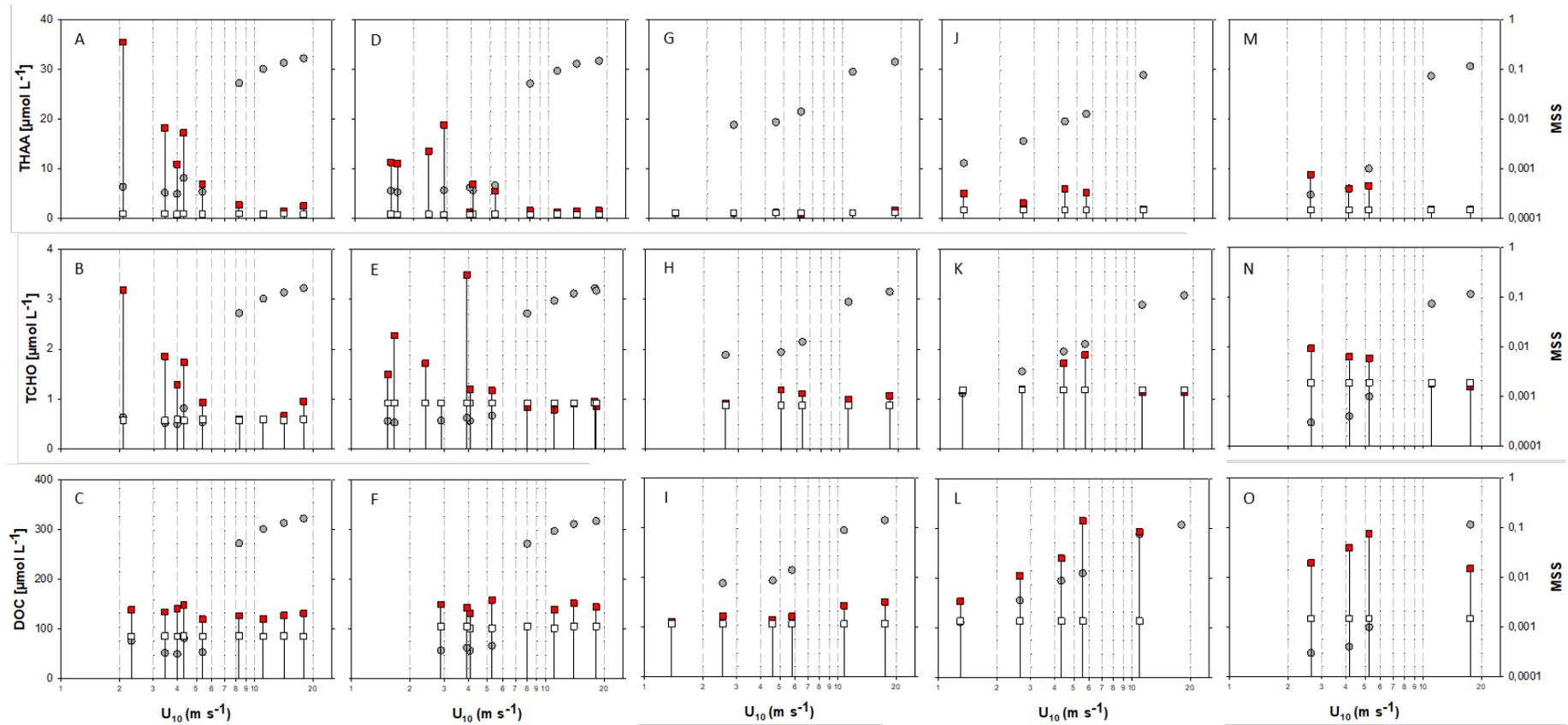
381

382 **Figure 4: Mean Square Slopes (MSS, dimensionless) relative to wind speed (U_{10} , $m s^{-1}$) during experiments**
 383 **with natural seawater. Days 2, 4, 9, 11 and 24 (red circles) with significant wave damping at wind speeds**
 384 **<6 $m s^{-1}$; day 15 (light grey circles), and day 22 (grey circles) with little wave damping compared to pure**
 385 **freshwater (asterisk).**

386 For a better representation of biopolymer accumulation in the SML at different wind speeds, we grouped data for
 387 days 2 and 4, and for days 9 and 11. Days 15, 22 and 24 showed different patterns and are shown individually. In
 388 accordance with previously observed enrichment patterns, concentrations of biopolymers in the SML declined
 389 with increasing wind speed, showing a pronounced step to lower values at wind speeds $> 5-6 m s^{-1}$. This effect
 390 was most evident for experiments days 2-11, having the highest initial SML biopolymer concentration (Figure 5A-
 391 O). At wind speed $>5-6 m s^{-1}$, THAA and TCHO concentrations in the SML were similar or equal to ULW
 392 concentration. This collapse of the biopolymeric SML enrichment coincided with the sudden and pronounced
 393 change in MSS. On day 15, biopolymer concentration in the SML was not different from the ULW at initially low
 394 wind speed. The absence of an organic SML enrichment on day 15 may be attributed to enhanced microbial
 395 decomposition and is supported by the amino-acid-based degradation index (DI), which was lowest on day 15,
 396 suggesting a high degree of degradation (Engel et al., 2018). In this sense, the slight increase of DOC with

397 increasing wind speeds on day 15 and even more pronounced on days 22 and 24, i.e., after the addition of
398 phytoplankton and phytoplankton-derived organic matter to the ULW, suggests that organic matter of the
399 underlying water enriched the SML again, likely due to enhanced mixing and rising of film-covered bubbles after
400 wave-breaking, which is an established mechanism discussed in literature (Blanchard, 1975; Stefan and Szeri,
401 1999; Sabbaghzadeh et al., 2017) (Figure 5I). On days 22 and 24, higher biopolymer concentrations in the SML
402 were observed again, likely due to the addition of organic matter from a phytoplankton culture (day 20) and slick
403 material from an earlier mesocosm study (day 21). Biopolymer concentration and enrichment, however, stayed
404 below values observed during the first two weeks of the experiment. Enrichment of the biopolymers THAA and
405 TCHO in the SML ranged between 0.74 and 38 for EF_{THAA} and between 0.70 and 5.77 for EF_{TCHO} and fell to values
406 ~ 1 at $U_{10} > 5\text{-}6 \text{ m s}^{-1}$ also.

407 Enrichment of DOC in the SML varied between EF_{DOC} 1.04 and 2.78 and was not directly related to wind speed.
408 In contrast to THAA and TCHO, DOC concentration in the SML remained higher than in the underlying bulk
409 seawater or even increased at increasing wind speed. Differences in DOC concentrations between SML and bulk
410 seawater were moderate during experiments days 2, 4, 9 and 11 (Figure 5C, F), lowest on day 15 (Figure 5I) and
411 highest for the experiments conducted after the addition of organic material (Figure 5L, O).

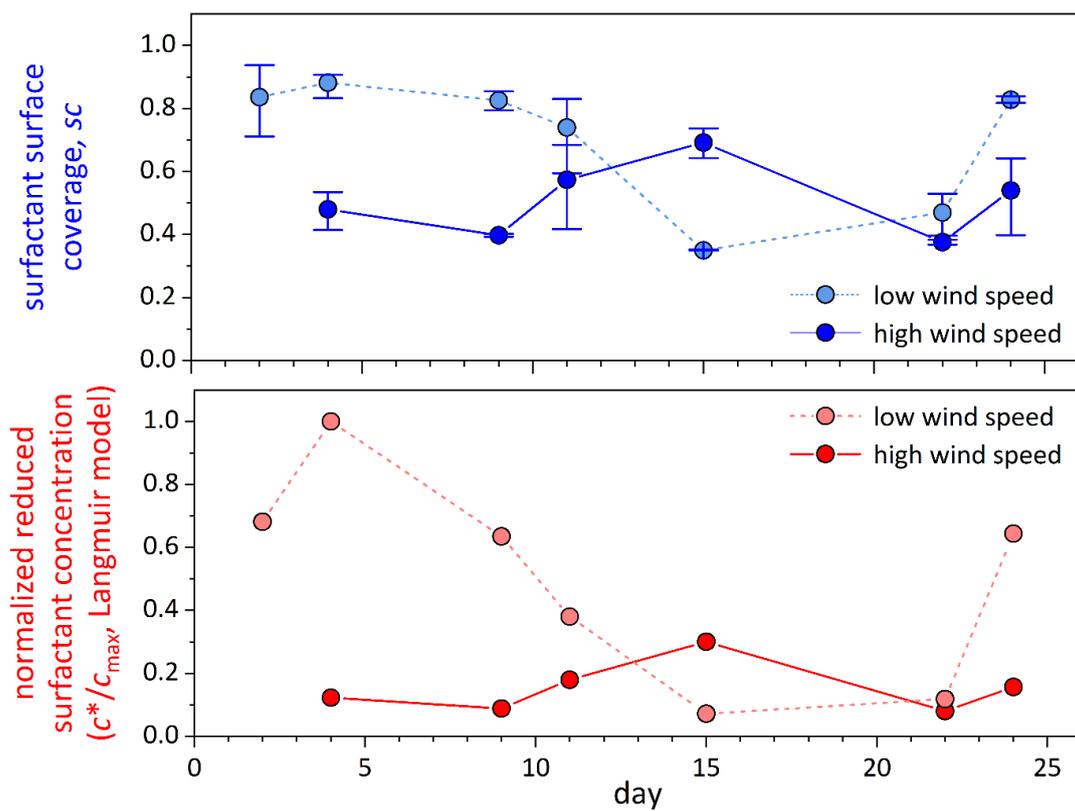


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413

414 **Figure 5A-O: Changes in organic matter components in the SML (red squares) and ULW (open squares) at different wind speeds (U_{10}), and associated MSS values (grey**
 415 **circles). For better coverage of wind speeds, samples with similarities in SML biopolymer concentrations and surface coverage were grouped, specifically samples were**
 416 **grouped for days 2 and 4: A-C and days 9 and 11: D-F. Day 15: G-I, day 22: J-L, day 24: M-O. Drop lines show associated SML, ULW pairs.**

417 Surfactant surface coverage and the corresponding reduced surfactant concentration in the bulk SML were
 418 determined only at the lowest and highest wind speeds, respectively (Figure 6). Both quantities were clearly
 419 reduced at high wind speed, except for day 15. As already outlined above, in contrast to all other days, day 15 did
 420 not show an enrichment of organics in the SML, along with a presumably high degree of degradation.
 421 Consequently, surfactant surface coverage closely resembled biopolymer accumulation in the SML. In general
 422 (excluding day 15), surfactant surface coverage (factor 1.6 ± 0.2 , $n = 5$) and effective surfactant concentration
 423 (factor 4.6 ± 1.5 , $n = 5$) were smaller at high wind speed and less variable at low wind speed, supporting the idea
 424 of surfactant accumulation in slicks.



425

426 **Figure 6: Surfactant surface coverage sc (blue symbols) and normalized reduced bulk SML surfactant**
 427 **concentration c^*/c_{max} (bars), as determined by VSFG spectroscopy for SML samples at the end of the**
 428 **lowest (light color) and highest wind speed (dark color) setting. No surfactant data were obtained at high**
 429 **wind on day 2.**

430

431

432

433 3.4 Wind-induced changes in biopolymer composition

434 In addition to concentration changes, wind speed also altered the monomeric composition of biopolymers in the
 435 SML. Wind speed clearly affected THAA composition in the SML, with a significant decrease in molar
 436 contributions of PHE, VAL, ARG, and ISO ($p<0.001$) and significant increases ($p<0.001$) in GLY, GABA, SER,
 437 and LEU, while changes were less or not significant for TYR, ALA, GLX, ASX, and THR (Table 1). At times of
 438 high THAA enrichment ($EF_{THAA}>6$), i.e. days 2-11, a strong selective enrichment of ARG and GLX was observed
 439 in the SML at all wind speeds, with EF_{Arg} being approximately twice as high as EF_{THAA} (Figure 7A). In contrast,
 440 GABA was relatively depleted in the SML, with EF_{GABA} being less than half as much as EF_{THAA} . Selective
 441 enrichment of ARG vanished after day 15 and was only slightly higher on days 22 and 24, with the highest
 442 $EF_{ARG}=6.8$ at $EF_{THAA}=5.3$.

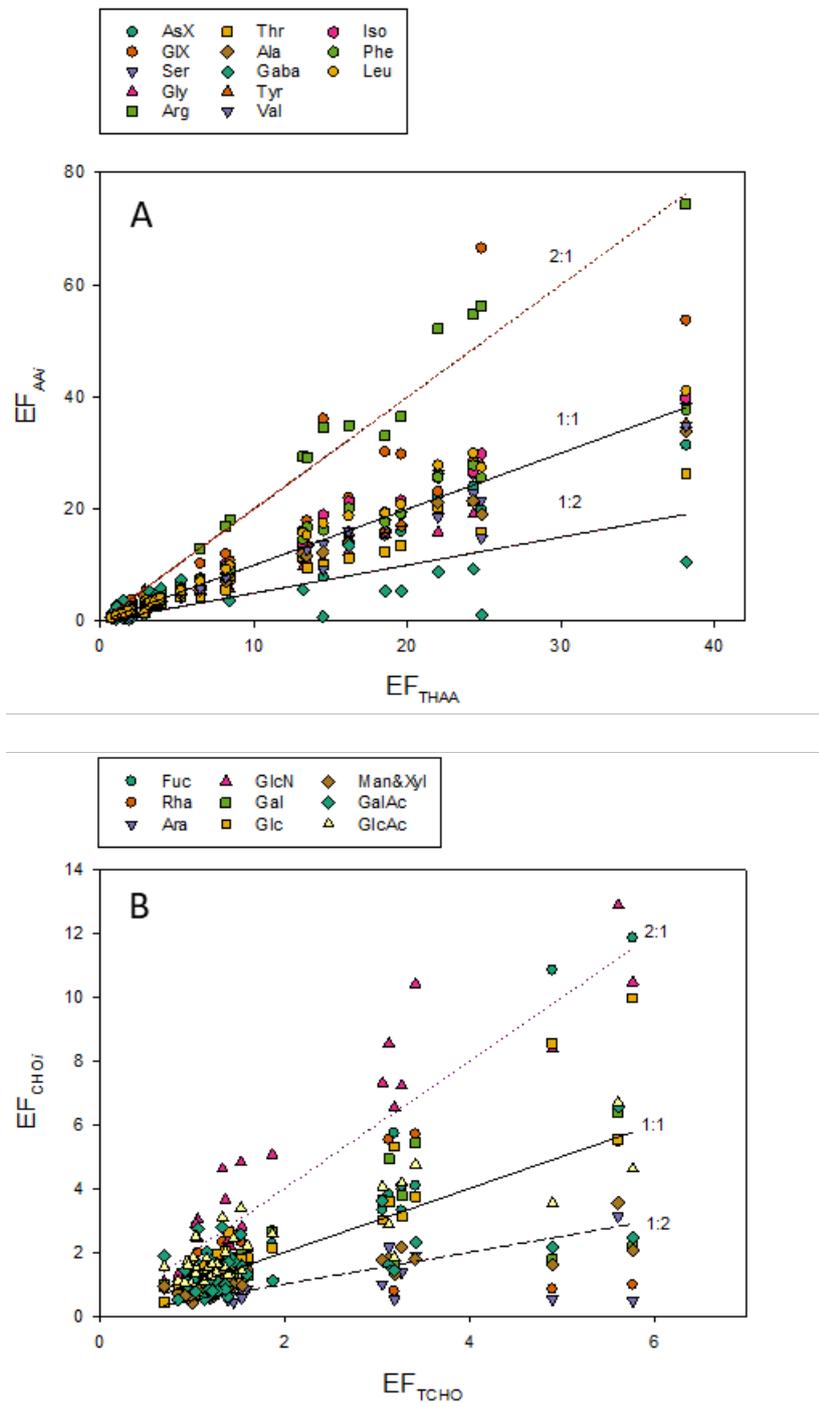
443 Wind-induced changes in THAA composition indicate that the rather fresh organic material that accumulated at
 444 the SML under low wind conditions was mixed into the underlying seawater and replaced at higher wind speeds
 445 by more diagenetically altered material. This was evident from the DI index being systematically higher at wind
 446 speed $<6\text{ m s}^{-1}$ than above (Figure 1S). Again, only day 15 stood out of this pattern with similarly low DI indices
 447 at all tested wind speeds. In contrast to THAA, wind-induced effects on the carbohydrate composition of
 448 biopolymers were less pronounced. A clearly significant selective decrease with increasing wind speed was
 449 observed for GLC-N, GAL and RHA (Table 1). Also, GAL became depleted at increasing winds, while no impact
 450 was observed on the uronic acids GLC-URA and GAL-URA, as well as on FUC. ARA was the only sugar that
 451 became clearly enriched in the SML with increasing wind speed, while GLC and XYL/MAN, being quantitatively
 452 the most important sugars, showed only a moderate relationship with wind speed.

453 Like individual amino acids, some sugars were selectively enriched in the SML, in particular when TCHO
 454 enrichment was relatively high ($EF_{TCHO}>2$) (Fig. 7B). This was most pronounced for the amino-sugar GAL-N with
 455 $EF_{GAL-N}:1.08-12.9$ compared to TCHO with $EF_{TCHO}:0.70-5.77$. Interestingly, only a slight selective enrichment
 456 was observed for the two uronic acids determined during this study when compared to EF_{TCHO} , i.e., GAL-URA
 457 ($EF_{GAL-URA}:0.51-6.57$) and GLC-URA ($EF_{GLC-URA}:1.04-6.57$). Uronic acids are building blocks of complex gel-
 458 like colloidal and particulate material suggested to form the SML (Sieburth, 1983; Cunliffe and Murrell, 2009),
 459 and accumulation of carbohydrate-rich gel-like transparent exopolymer particles (TEP) was also observed during
 460 this study (Sun et al., 2018). ARA and XYL/MAN were consistently less enriched than TCHO, showing
 461 $EF_{Ara}:0.44-3.12$ and $EF_{XYL/MAN}:0.41-3.55$, respectively.

462 **Table 1: Correlation between wind speed (U_{10}) and monomeric components (Mol%) of TCHO (left column)**
 463 **and THAA (right column) as observed for the SML samples. n.s.: not significant.**

	<i>r</i>	<i><p</i>		<i>r</i>	<i><p</i>
GLC-N	-0.761	0.001	PHE	-0.885	0.001
RHA	-0.687	0.005	VAL	-0.774	0.001
GAL	-0.425	0.01	ARG	-0.705	0.001
FUC	-0.216	n.s.	ISO	-0.675	0.005
GLC-URA	-0.197	n.s.	TYR	-0.389	0.01
GAL-URA	-0.077	n.s.	ALA	-0.34	0.01
GLC	0.419	0.01	GLX	-0.339	0.01
XYL/MAN	0.457	0.01	ASX	-0.203	n.s.
ARA	0.731	0.001	THR	0.0956	n.s.
			GLY	0.645	0.001
			GABA	0.674	0.001
			SER	0.712	0.001
			LEU	0.836	0.001

464



465

466 **Figure 7A, B: Relationships between Enrichment Factors (EFs) of individual amino acids (EF_{AAi}) and total**
 467 **hydrolysable amino acids (EF_{THAA}) (A) and between EFs of individual sugars (EF_{CHOi}) and total combined**
 468 **carbohydrates (EF_{TCHO}) (B). Lines shown for reference: 2-fold enrichment (2:1), no enrichment (1:1) and**
 469 **2-fold depletion (1:2).**

470

471

472 4. Discussion

473 4.1 Accumulation of biopolymers at the air-sea interface

474 Seven experiments were conducted with natural seawater in the annular wind-wave channel *Aeolotron* and
475 revealed distinct patterns regarding the accumulation of natural organic matter in the SML, the impact of wind
476 speed on biopolymer enrichment and composition, as well as the effects of biopolymer enrichment on capillary
477 wave damping. Firstly, biopolymers, specifically substances containing amino acids and carbohydrates, were
478 found to be highly enriched in the SML at low wind speeds ($<6 \text{ m s}^{-1}$).

479 Biopolymers have long been considered important in the SML dynamics; the SML itself proposed to be a highly
480 hydrated loose gel of tangled macromolecules and colloids (Sieburth, 1983; Cunliffe and Murrell, 2009). During
481 this study, the range of biopolymer (THAA + TCHO) concentration in the SML was $1.4\text{-}40 \mu\text{mol L}^{-1}$, which is
482 comparable to the range observed in the ocean. For instance, average SML concentrations of $1.72 \pm 0.44 \mu\text{mol L}^{-1}$
483 TAA and $1.1 \pm 0.49 \mu\text{mol L}^{-1}$ TCHO were determined in the tropical Eastern North Atlantic (Barthelmeß et al.,
484 2021) and approximately $2 \mu\text{mol L}^{-1}$ THAA and $2.5\text{-}3.8 \mu\text{mol L}^{-1}$ TCHO were found in the western Baltic Sea
485 (Barthelmeß and Engel, 2022). In the highly productive upwelling system off Peru, SML concentrations can reach
486 up to $6 \mu\text{mol L}^{-1}$ THAA and $7.8 \mu\text{mol L}^{-1}$ TCHO (Engel and Galgani, 2016). For the North-Western Atlantic
487 Ocean, THAA concentrations of up to $10 \mu\text{mol L}^{-1}$ have been reported (Kuznetsova et al., 2004). Likewise, variable
488 and high enrichments of biopolymers in the SML have been observed. For instance, EFs of dissolved amino acids
489 varied between 5 and 43 in the subtropical Atlantic and Mediterranean Seas (Reinthal et al., 2008) and between
490 1.1 and 9 in the Eastern Tropical South Pacific (Zäncker et al., 2017). As observed during this study, the enrichment
491 of amino acids in the SML often exceeds the enrichment in carbohydrates (Engel and Galgani, 2016; Zäncker et
492 al., 2017; van Pinxteren et al., 2012).

493 Based upon high biopolymer enrichment, the SML often shows typical biofilm properties (Wurl and Homes,
494 2008), with high biological activity and specifically adapted organisms, i.e., neuston. Hydrolysis experiments
495 revealed that microbial activity can significantly reduce amino acid concentrations in microlayer samples, even to
496 values below those found in the underlying water (Kuznetsova and Lee, 2001). During this study, the amino acid
497 and carbohydrate concentrations in the SML were reduced to the ULW level by day 15, indicating that microbial
498 degradation may indeed counteract biopolymer accumulation and, therefore, slick formation in the sea.

499

500 **4.2 Interactions of wind speed and biopolymer accumulation in the SML**

501 The conditions in the *Aeolotron* at low wind speeds resembled typical slick conditions as observed in the field.
502 The most prominent property of surfactants is their damping of capillary waves, as indicated by a reduction in the
503 MSS value. The damping effect results from the dissipation of wave energy due to changes in the viscoelasticity
504 of the interfacial surface layer (Cini et al., 1983) and is referred to as the Marangoni effect (McKenna and Bock,
505 2006). The intensity of the Marangoni effect depends on the quantity and composition of surface-active compounds
506 in slicks. Under slick conditions, accompanied by high values of surfactant coverage, MSS values in the *Aeolotron*
507 were reduced by about 1-2 orders of magnitude compared to the freshwater reference. However, the damping
508 effect largely vanished at $>6 \text{ m s}^{-1}$. At a wind-speed threshold of approximately 6 m s^{-1} , the collapse of the
509 biopolymeric surface layer induced an abrupt change in sea-surface roughness. Because the MSS is widely
510 recognized as a predictor of air-sea gas transfer velocity (McKenna and Bock, 2006; Frew et al., 2004), such an
511 abrupt shift in surface roughness should likewise be reflected in the gas-transfer measurements. Indeed, Ribas-
512 Ribas et al. (2018) reported a decrease in N_2O gas transfer velocities at wind speeds of approximately $U_{10} = 5.5\text{--}8$
513 m s^{-1} , during an accompanying *Aeolotron* experiment—findings that are highly consistent with our observations.

514 Previous wind-wave tank experiments, not carried out on natural samples but with strong artificial surfactants,
515 showed significant wave damping until wind speeds of $U_{10} \sim 18 \text{ m s}^{-1}$ (Alpers and Hühnerfuss, 1989). During
516 experiments in a linear wind-wave tunnel, an artificial surface film (oleyl alcohol) began to tear at a wind speed
517 of 13 m/s (Broecker et al., 1978). Previous *Aeolotron* experiments with surface films of hexadecanol and olive oil
518 and with the soluble surfactants Triton X-100 and Tergitol 15-S-12 at a concentration of 5 ppm also showed
519 damping effects at higher wind speed (Jähne, *unpublished*). So far, wind-wave tank experiments with natural
520 seawater and, hence, natural surfactants and surface films remain scarce. Our data show that the wave-damping
521 effects of biogenic surface films may behave differently from artificial films. Natural SML components may be
522 more susceptible to wind-induced disruption and more variable over time and space. Biopolymer variability in the
523 SML may thus be expected over the diurnal cycle, as wind speed often increases during the night. Our data also
524 show that the amount and chemical composition of biopolymers and, in consequence, the surface activity can vary
525 with microbial production and decomposition. Due to natural chemical heterogeneity, the impact of natural surface
526 film on air-sea gas exchange, however, may differ from our observations. In particular, where stronger surfactants
527 are present, also natural films may resist higher wind speeds. For instance, surfactant enrichment in the SML has
528 been reported for wind speeds of up to 9.5 m s^{-1} (Wurl et al., 2011); DOC enrichment up to 9.7 m s^{-1} in the
529 Mediterranean Sea (Reinthal et al., 2008), and enrichment of combined amino acids in the North Atlantic Ocean

530 at wind speeds of 7 m s^{-1} (Kuznetsova et al., 2004). Clearly, a mechanistic understanding of wind speed and SML
531 biopolymer enrichment has yet to be established. In this regard, conducting controlled wind-wave experiments
532 using natural seawater can offer important insights into the role of natural surfactants in modulating air-sea gas
533 exchange.

534 On the one hand and in contrast to THAA and TCHO biopolymers, no significant relationship between wind speed
535 and DOC enrichment was observed. On the other hand, the apparent thickness of the SML increased significantly
536 with rising wind speeds. The sensitivity of SML thickness to wind speed has been reported previously, with an
537 increase in apparent SML thickness up to wind speeds between 5.5 and 7.9 m s^{-1} (Beaufort 4) in the Baltic Sea
538 (Falkowska, 1999), or a decrease in SML thickness with wind speeds ranging from 1 to 5 m s^{-1} (Liu and Dickhut,
539 1998). This may be explained by different and partly antagonistic processes influencing organic matter enrichment
540 in the SML. On the one hand, wind can reduce microlayer enrichment through turbulent mixing, which increases
541 with wind speed. Conversely, wind can enhance the enrichment of certain components by promoting bubble
542 formation, which facilitates the scavenging of organic matter from the ULW to the microlayer (Hunter and Liss,
543 1981). In the environment, other processes also interact with organic matter enrichment, such as the production or
544 decomposition of organic matter in the SML or the mixing and advection of water masses. Our study allowed us
545 to follow the changes in SML composition with increasing wind speed and suggests that the enrichment of organic
546 matter in the SML and its response to wind is highly compound-specific. Biopolymers, i.e. TCAA and TCHO,
547 showed the highest EFs and responded similarly to increasing wind, with no discernible difference between SML
548 and underwater concentrations at wind speed $> 6 \text{ m s}^{-1}$. DOC is a bulk measure and includes a variety of different
549 substances that may be more or less prone to mixing or enrichment. Indeed, EFs for DOC were rather low
550 compared to THAA and TCHO. DOC concentration in the SML may simply be high because of diffusive exchange
551 with high background concentration of organic substances do not have surfactant properties. Hence, a uniform
552 relationship of DOC enrichment in the SML with regard to wind speed seems unlikely. In this study, DOC
553 concentration and enrichment in the SML increased with wind speed on day 15 and were even more pronounced
554 on days 22 and 24. This indicates a net upward transport of DOC from the ULW to the SML due to increased
555 turbulence or rising bubbles at higher wind speeds. Because of the high concentration of DOC, an increase in DOC
556 may have contributed to the increase in apparent thickness (d) of the SML with wind speed observed during this
557 study, although it cannot fully explain it. This also shows that apparent SML thickness and visually apparent slick
558 conditions are not necessarily related.

559 During the first two weeks in the *Aeolotron*, slicks showed THAA accumulation up to 10 times higher than TCHO
560 accumulation. This aligns with observations that protein-rich, gel-like particles were highly enriched at low wind
561 speeds, especially in the early stages of the experiment (Sun et al., 2018). At the same time, the highest surfactant
562 coverage, as determined by VSFG-spectroscopy, was observed. The [THAA]:[TCHO] or, more generally, the
563 protein/carbohydrate (P/C) ratio in the SML was highest on days 2 and 4. This suggests that polypeptides not only
564 played an important role in slick formation but also included particularly powerful biosurfactants. The P/C ratio
565 of biopolymers has been interpreted as an indicator for the relative hydrophobicity of extracellular polymeric
566 substances (EPS) (Santschi et al., 2020), based on observations of increasing hydrophobic contact area (HCA)
567 with increasing P/C ratio (Xu et al., 2011). Exopolymers with high P/C ratios are mainly produced by bacteria,
568 whereas phytoplankton EPS contain more carbohydrates (Santschi et al., 2020). The high P/C ratio of surface
569 slicks at the beginning of this study can be explained by the predominance of bacterial biomass in the seawater,
570 which was collected in the deep North Sea and not exposed to light until day 8 of the experiment. Our observations
571 showed that capillary waves were most strongly damped on days 2-11. In contrast, the P/C ratio was much lower,
572 with values ~ 4 , when SML material from phytoplankton origin replenished the organic matter pool of the SML on
573 days 22 and 24. Since the seawater, which was used to fill the *Aeolotron*, had been stored in the dark for about one
574 month prior to the experiment, any fresh material must have been derived mainly from heterotrophic bacterial
575 production. The high P/C ratio, together with the high DI value of the organic material in the SML at the beginning
576 of the study, suggests that bacterial-derived biopolymers accumulate and act as powerful biosurfactants in the
577 SML.

578 Within the pool of THAA and THCO, monomers with dielectric properties (basic/acidic AAs and basic/acidic
579 CHO) were most enriched and most sensitive to wind speed, suggesting that surfactant properties are linked to
580 those monomeric components. Among the amino acids, significantly enriched in the SML were GLX and ARG.
581 High GLX and ARG enrichment has been reported for oceanic SML previously (Barthelmeß et al., 2021; Sun et
582 al., 2018; van Pinxteren et al., 2012). In general, the enrichment of amino acids at the air-sea interface depends on
583 their amphiphilic properties (Ćosović and Vojvodić, 1998), which arise from the degree of polarity exhibited on
584 their molecular surfaces. Among the amino acids discussed here, ARG and GLX are considered hyperpolar and
585 represent typical hydrophilic head groups of biosurfactants. Lipoamino acids derived from ARG have increasingly
586 gathered interest in biotechnological applications as they represent nontoxic and degradable cationic biosurfactants
587 with anti-microbial properties (Singh and Tyagi, 2014). Surfactant activity in surface waters of the Tropical
588 Eastern North Atlantic has been directly related to ARG concentration (Barthelmeß et al., 2011). The GLX-

589 containing lipopeptide *Surfactin*, produced by *Bacillus spp.*, a species also found in seawater, is one of the most
590 effective biosurfactants (Zhen et al., 2023). During this study, we didn't identify the molecular structure of
591 surfactants. However, the high THAA enrichment in the SML, together with even higher selective enrichment of
592 ARG and GLX, and strong surfactant activity, point to lipoamino acids with a bacterial source during the first days
593 of this study.

594 Enrichment in the SML was generally smaller for TCHO than for THAA. On day 22, after material from a
595 phytoplankton culture and bloom experiment was added, and the P/C ratio in the SML decreased, MSS values at
596 comparable wind speeds were higher. Marine photoautotrophic plankton is the major source of biomolecules in
597 the ocean, providing ~50 Gt of organic carbon yr⁻¹. In general, the biochemical composition of autotrophic cells
598 comprises the following major components by weight: proteins (17–57%), carbohydrates (4.1–37%), and lipids
599 (2.9–18%) (Parsons et al., 1961). Extracellular polymers released from the autotrophic cell, however, contain
600 largely polysaccharides (Engel et al., 2004; Thornton, 2014). Among the carbohydrates that showed a selective
601 enrichment in the SML was FUC, a sugar that is typically found in polysaccharides released from phytoplankton
602 and seaweeds (Buck-Wiese et al., 2023), and GLC-N, a sugar contained in bacterial exopolymers (Maßmig et al.,
603 2024). GLC-N, RHA and Gal were particularly sensitive to wind speed. GLC-N is often contained in biosurfactants
604 and, like arginine, has received attention in the biotechnological search for replacement of toxic synthetic
605 surfactants. Rhamnolipids are typical biosurfactants consisting of one or two rhamnose sugar molecules linked to
606 hydroxy fatty acid chains. Galactolipids can be found in some cyanobacteria and algae and include galactose
607 residues linked to lipid moieties. However, compared to peptide-based surfactants, carbohydrate-based surfactants
608 seem to be less abundant or less effective during this study.

609 The amino-acids-based DI, as well as the presence of FUC, suggested that organic matter accumulating at the SML
610 was less degraded than in the underlying seawater. This finding is consistent with earlier findings on SML
611 biopolymer composition and surfactant activity in the Baltic Sea (Barthelmeß and Engel, 2022), which show that
612 the highest surface activity was triggered by the microbial release of fresh organic matter. Marine microorganisms
613 release surfactants for several ecological and physiological reasons. For example, they act as emulsifiers and aid
614 in substrate uptake, in particular hydrophobic organic compounds, such as oil, and are produced by a variety of
615 marine bacteria (Floris et al., 2020). Moreover, surfactants facilitate the colonization of surfaces by helping
616 microorganisms adhere to substrates and form biofilms. In higher organisms, e.g., mammals, surfactants are critical
617 to maintaining lung function or for skin protection. A common feature of surfactants is their accumulation at
618 interfaces. The air-sea interface, including both the SML and bubbles, represents the largest interface in the ocean

619 and serves as a trap for surfactants released to seawater. Since microbial surfactants are used extracellularly, they
620 must be stable enough in the marine environment to fulfil their ecological roles. The production and subsequent
621 accumulation of biopolymers, including surfactants, in the SML illustrate how marine life can alter the physical
622 environment at the ocean's surface. This biotic effect on upper ocean physics has direct implications for climate
623 regulation, as changes in gas exchange and surface turbulence can impact the ocean's role in sequestering carbon
624 dioxide and regulating atmospheric gases. Thus, these effects may be particularly pronounced in areas of high
625 surfactant productivity, highlighting the complex interplay and feedback between biodiversity, chemical diversity,
626 and air-sea exchange in the ocean.

627 Our research revealed that biopolymers, particularly polypeptides, produced by marine microorganisms, serve as
628 efficient natural surfactants in the SML. Natural surfactants that accumulated in the SML during this study
629 exhibited a significant damping effect on wave formation up to wind speeds of $U_{10} \approx 6 \text{ m s}^{-1}$. However, at even
630 higher wind speeds and going along with the collapse of the biopolymeric SML, the damping effect largely
631 vanished. This sheds light on the ecological role of marine biopolymers and underscores their influence on physical
632 air-sea exchange processes. A better understanding of the dynamic linkages between marine life and gas exchange
633 could be pivotal to accurately assessing the ocean's present and future contributions to the climate system,
634 including the uptake or release of climate-relevant gases like CO_2 and methane.

635

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645 enrichment processes of organic matter in the SML and 1.4 Chemical and photochemical transformation of organic
646 matter.

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